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Parameter-robustness analysis for a biochemical oscillator model describing the social-behavior transition phase of *myxobacteria*

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We develop a tool based on bifurcation analysis for parameter-robustness analysis for a class of oscillators and in particular, examine a biochemical oscillator that describes the transition phase between social behaviors of myxobacteria. Myxobacteria are a particular group of soil bacteria that have two dogmatically different types of social behavior: when food is abundant they live fairly isolated forming swarms, but when food is scarce, they aggregate into a multicellular organism. In the transition between the two types of behaviors, spatial wave patterns are produced, which is generally believed to be regulated by a certain biochemical clock that controls the direction of myxobacteria's motion. We provide a detailed analysis of such a clock and show that, for the proposed model, there exists some interval in parameter space where the behavior is robust, i.e., the system behaves similarly for all parameter values. In more mathematical terms, we show the existence and convergence of trajectories to a limit cycle, and provide estimates of the parameter under which such a behavior occurs. In addition, we show that the reported convergence result is robust, in the sense that any small change in the parameters leads to the same qualitative behavior of the solution.

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1. Introduction

Oscillators as theoretical models capture various oscillating behaviors in dynamical processes that have been studied in engineering [1], biology [2], neuroscience [3], medicine [4,5], biochemistry [6,7], and other scientific fields. In this article, we investigate a biochemical oscillator that describes the behavior of *myxobacteria* during their development of a multicellular structure [8]. Myxobacteria are found in the soil and have multicellular social behavior. They live as a unicellular organism, which, as long as food is abundant, propel themselves towards the formation of small swarms by a mechanism called "gliding" [8]. However, whenever food is scarce, they aggregate and initiate a complex developmental program that transforms the swarms into a multicellular single body, called "fruiting body" [9]; in the transformation, while the fruiting body is forming, myxobacteria pass through a developmental stage, called the "ripple phase" [8], which is characterized by elaborate spatial wave patterns propagating within the whole colony. It has been observed that different myxobacteria communicate with each other by direct cell contacts. Thus, the waves are produced by the back and forth motion of the bacteria. More specifically, these wave patterns are created through motion coordination using the so called "C-signaling", which is a contact dependent signal that influences how often the bacteria reverse their motion directions. It is through the combination of the reversal times that myxobacteria produce the observed complicated wave patterns. Thus, in [8], a "clock" that controls the reversals has been suggested in the form of a biochemical oscillator model.

This oscillator is described by a three-dimensional ordinary differential equation, which will be further described in Section 2. From observations based on numerical simulations, it has been argued that the model is robust [8]. In particular, it has been argued that the overall behavior of the oscillator remains the same upon small variation of parameters. Correspondingly, the main contribution of this paper is to formalize the above claims by means of rigorous mathematical bifurcation analysis. More precisely, we prove that there exists an open set of parameter values, under which the model is robust, and more importantly, we provide an estimate of such an interval. Furthermore, we show that for almost all initial conditions, and a certain range of parameter values, the trajectories converge to a finite number of periodic solutions, at least one of which is asymptotically stable. With these results we rule out the existence of chaotic and homoclinic solutions for the identified parameter interval. We emphasize that the methods and techniques used in this paper are not confined to the analysis of the particular myxobacteria model, but rather applicable to a wide range of systems having oscillatory behavior.

The rest of this paper is arranged as follows. In Section 2 we provide a detailed description of the biochemical oscillator model. Next, in Section 3 we develop local analysis and show that the dynamics of the system can be studied under a perturbation framework. Afterwards, in Section 4 we give sufficient conditions for the existence of periodic solutions using Hopf bifurcation theory. In Section 5 global analysis is carried out to study the convergence of trajectories to periodic solutions. The robustness of the convergence behavior is presented in Section 6. We end this paper in Section 7 with conclusions and a summary on open problems.

2. System description

We study a mathematical model that describes several important properties of myxobacteria during development [8]. This model, so-called the Frz system, is based on a negative feedback loop. The Frz system includes a methyltransferase (FrzF), the cytoplasmic methyl-accepting protein (FrzCD), and a protein kinase (FrzE). When two cells of myxobacteria collide with each other *directly by end-to-end*, a C-signal is produced. After the C-signal transmission, a protein called FruA is phosphorylated. The signal from phosphorylated FruA (FruA-P) activates the Frz proteins as follows: (i) FruA-P activates the methyltransferase FrzF (x_1); (ii) FrzCD is methylated in response to x_1; (iii) the methylated form of FrzCD (x_2) influences the phosphorylation of FrzE; and (iv) the phosphorylated form of FrzE (x_3) inhibits x_1. A schematic representation of the Frz

system is shown in Figure 1. For a more detailed explanation of the model and its biological background, see [8]. The interaction between x_1, x_2 and x_3 is modeled by Michaelis-Menten kinetics, which leads to the following dynamical system

$$\begin{aligned} x_1' &= k_{\mathrm{a}}(1-x_1) - k_{\mathrm{d}}x_1x_3, \\ x_2' &= k_{\mathrm{m}}(1-x_2)x_1 - k_{\mathrm{dm}}x_2, \\ x_3' &= k_{\mathrm{p}}(1-x_3)x_2 - k_{\mathrm{dp}}x_3, \end{aligned} \tag{2.1}$$

where

$$\begin{aligned} k_{\mathrm{a}} &= \frac{k_{\mathrm{a}}^{\max}}{K_{\mathrm{a}}+(1-x_1)}, & k_{\mathrm{d}} &= \frac{k_{\mathrm{d}}^{\max}}{K_{\mathrm{d}}+x_1}, \\ k_{\mathrm{m}} &= \frac{k_{\mathrm{m}}^{\max}}{K_{\mathrm{m}}+(1-x_2)}, & k_{\mathrm{dm}} &= \frac{k_{\mathrm{dm}}^{\max}}{K_{\mathrm{dm}}+x_2}, \\ k_{\mathrm{p}} &= \frac{k_{\mathrm{p}}^{\max}}{K_{\mathrm{p}}+(1-x_3)}, & k_{\mathrm{dp}} &= \frac{k_{\mathrm{dp}}^{\max}}{K_{\mathrm{dp}}+x_3}. \end{aligned} \tag{2.2}$$

The schematic diagram of model (2.1) is presented in Figure 1.

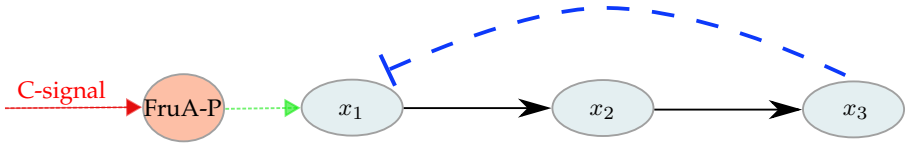


Figure 1: The schematic diagram of system (2.1). The feed-forward (or activation) and the negative feedback (or inhibition) are shown respectively by $\rightarrow$ and $\vdash$. The FruA-P signal that activates x_1 is constant.

In [8], the choices of the parameter values are as follows. First, the C-signal, denoted by $k_{\mathrm{a}}^{\max}$, is assumed to be constant. Next, the following parameter values are given $K_{\mathrm{a}} = 10^{-2}$, $K_{\mathrm{d}} = K_{\mathrm{m}} = K_{\mathrm{dm}} = K_{\mathrm{p}} = K_{\mathrm{dp}} = 5\times 10^{-3}$, $k_{\mathrm{d}}^{\max} = 1$ min^{-1}, $k_{\mathrm{m}}^{\max} = k_{\mathrm{p}}^{\max} = 4$ min^{-1}, $k_{\mathrm{dm}}^{\max} = k_{\mathrm{dp}}^{\max} = 2$ min^{-1}, and $k_{\mathrm{a}}^{\max} = 0.08$ min^{-1}. It is observed *numerically* in [8] that under these parameter values, system (2.1) exhibits oscillatory behavior. Note that the reaction possesses the property of "zero-order ultrasensitivity" [8], meaning that the Michaelis-Menten constants $K_{\mathrm{a}}, K_{\mathrm{d}}, K_{\mathrm{m}}, K_{\mathrm{dm}}, K_{\mathrm{p}}$ and K_{dp} have to be *small* [10]. Since $K_{\mathrm{a}}, K_{\mathrm{d}}, K_{\mathrm{m}}, K_{\mathrm{dm}}, K_{\mathrm{p}}$ and K_{dp} are dimensionless Michaelis-Menten constants, we propose to set $K_{\mathrm{a}} = 2K_{\mathrm{d}} = 2K_{\mathrm{m}} = 2K_{\mathrm{dm}} = 2K_{\mathrm{p}} = 2K_{\mathrm{dp}} = \varepsilon$. We remark, however, that although $k_{\mathrm{a}}^{\max}$ is small as well, its unit is "min^{-1}" which cannot be unified with the Michaelis-Menten constants. Substituting (2.2) in (2.1), and taking care of the previous considerations, we obtain the following dynamical system

$$\begin{aligned} x_1' &= \frac{0.08(1-x_1)}{\varepsilon+(1-x_1)} - \frac{2x_1x_3}{\varepsilon+2x_1}, \\ x_2' &= \frac{8(1-x_2)x_1}{\varepsilon+2(1-x_2)} - \frac{4x_2}{\varepsilon+2x_2}, \\ x_3' &= \frac{8(1-x_3)x_2}{\varepsilon+2(1-x_3)} - \frac{4x_3}{\varepsilon+2x_3}. \end{aligned} \tag{2.3}$$

For the sake of brevity, we denote system (2.3) by

$$x' = G(x,\varepsilon), \tag{2.4}$$

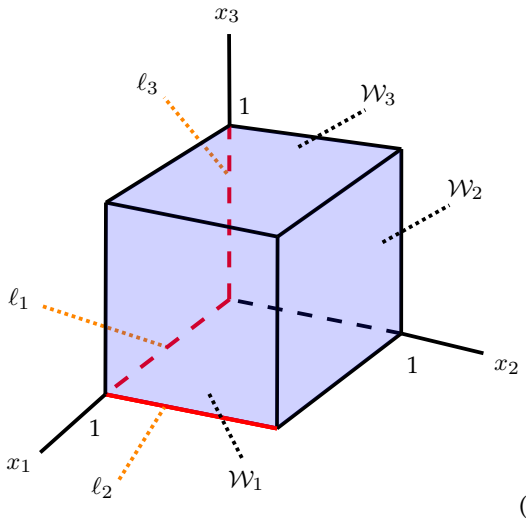
where $x = \begin{bmatrix} x_1, & x_2, & x_3 \end{bmatrix}^\top$, $G(x,\varepsilon) = \begin{bmatrix} G_1(x,\varepsilon), & G_2(x,\varepsilon), & G_3(x,\varepsilon) \end{bmatrix}^\top$, and $G_i(x,\varepsilon)$ are the right-hand sides of x_i' in (2.3) for $i = 1, 2, 3$.

Remark 2.1. *From a biochemical point of view, the variables x_1, x_2, x_3 of system (2.3) stand for fractions of activated protein concentrations. Therefore, their values are restricted to $[0, 1]$. Thus, from now on, we confine our analysis to the unit cube (see Figure 2a) defined by*

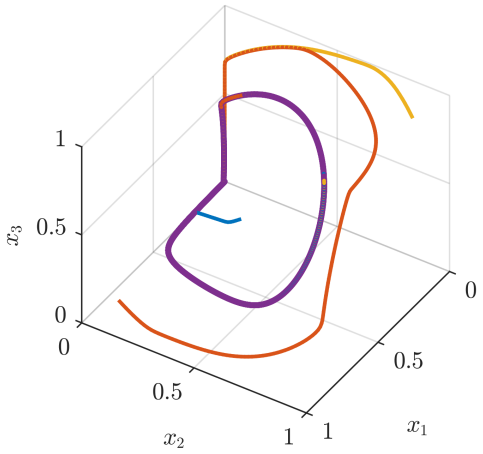
$$\mathcal{C} := \left\{ x \in \mathbb{R}^3 \mid x \in [0,1] \times [0,1] \times [0,1] \right\}.$$

Our numerical simulations (see Figure 2b) show that system (2.3) has the following characteristics:

- the trajectories are contained in the unit cube $\mathcal{C}$ provided that they start within,
- for the particular value $\bar{\varepsilon} = 0.01$ used in [8], and in general, for a *sufficiently small* perturbation of $\bar{\varepsilon}$, the solutions are periodic,
- the solutions converge to a periodic limit cycle.



(a) The unit cube $\mathcal{C}$.



(b) Trajectories of system (2.3) for $\varepsilon = 0.01$ with three different initial conditions.

Figure 2

Due to the fact that these three properties are highly interesting, because of their biological implications for understanding the developmental stage of maxobacteria, it is of great importance to provide rigorous mathematical analysis in addition to the simulation results reported so far. More precisely, since the Michaelis-Menten constants have not been experimentally identified [8], it is crucial to be able to predict the range of parameters under which the model produces the anticipated oscillatory behavior for which it has been designed. Towards this goal, we start our analysis of (2.3) by investigating its local properties in the next section.

3. Local analysis

Some of the arguments that we use in this paper are of a "regular perturbation" nature. Therefore, before providing any details, we show that the *local* properties around a unique equilibrium point of the vector field $x' = G(x, \varepsilon)$ can be regarded as a regular perturbation problem of $x' = G(x, 0)$ for "sufficiently small" $\varepsilon > 0$. More specifically, we show that $x' = G(x, 0)$ is structurally stable near its equilibrium point, see Definition 3.3 below.

Definition 3.1 (C^1 ε-perturbation [11]). *Consider two vector fields F_1 and F_2 on $\mathbb{R}^n$. We say that F_2 is a C^1 ε-perturbation of F_1 in a closed region $\mathcal{U} \subset \mathbb{R}^n$ if*

$$d := \sup_{x \in \mathcal{U}} \left\{ \|F_1(x) - F_2(x)\| + \left\| \frac{\partial F_1(x)}{\partial x} - \frac{\partial F_2(x)}{\partial x} \right\| \right\} \leq \varepsilon, \tag{3.1}$$

where $\|\cdot\|$ is an arbitrary norm in $\mathbb{R}^n$.

Note that the distance d is zero if $F_1(x) = F_2(x)$ for $x \in \mathcal{U}$.

Definition 3.2 (Topological equivalence [12]). *Two vector fields F_1, F_2 on $\mathbb{R}^n$ are said to be topologically equivalent if there exists a homeomorphism $h : \mathbb{R}^n \longrightarrow \mathbb{R}^n$ which takes trajectories of F_1 to trajectories of F_2, preserving senses but not necessarily parametrization by time.*

Definition 3.3 (Structural stability [12]). *A vector field F on $\mathbb{R}^n$ is called structurally stable if there is an $\varepsilon > 0$ such that all C^1 ε-perturbations of F are topologically equivalent to F.*

Hereafter, the interior and the boundary of a set $\mathcal{S} \subset \mathbb{R}^n$ are respectively denoted by $\mathring{\mathcal{S}}$ and $\partial\mathcal{S}$. We denote the boundary of the cube $\mathcal{C}$ by $\partial\mathcal{C} := \bigcup_{i=1}^{6} \mathcal{W}_i$ where (see Figure 2a)

$$\begin{aligned} &\mathcal{W}_1 := \{x \in \mathcal{C} \,|\, x_1 = 1\}, \quad &&\mathcal{W}_2 := \{x \in \mathcal{C} \,|\, x_2 = 1\}, \quad &&\mathcal{W}_3 := \{x \in \mathcal{C} \,|\, x_3 = 1\}, \\ &\mathcal{W}_4 := \{x \in \mathcal{C} \,|\, x_1 = 0\}, \quad &&\mathcal{W}_5 := \{x \in \mathcal{C} \,|\, x_2 = 0\}, \quad &&\mathcal{W}_6 := \{x \in \mathcal{C} \,|\, x_3 = 0\}. \end{aligned} \tag{3.2}$$

The following lemma shows that for any $\varepsilon > 0$, the equation $G(x, \varepsilon) = 0$ does not have any solution on the boundary of $\mathcal{C}$.

Lemma 3.1. *For any $\varepsilon > 0$, the boundary of the cube $\mathcal{C}$ does not contain any equilibria of system (2.3).*

Proof. Let us show only one case on the wall $\mathcal{W}_1 = \{x \in \mathcal{C} \,|\, x_1 = 1\}$, which exemplifies the situation for the rest of the walls. Restricted to $\mathcal{W}_1$, system $x' = G(x, \varepsilon)$ reads as

$$x_1' = -\frac{2x_3}{\varepsilon + 2} \tag{3.3a}$$

$$x_2' = \frac{8(1 - x_2)}{\varepsilon + 2(1 - x_2)} - \frac{4x_2}{\varepsilon + 2x_2} \tag{3.3b}$$

$$x_3' = \frac{8(1 - x_3)x_2}{\varepsilon + 2(1 - x_3)} - \frac{4x_3}{\varepsilon + 2x_3}. \tag{3.3c}$$

If $(1, x_2^*, x_3^*)$ is an equilibrium point of (3.3) then, from (3.3*a*) we have that necessarily $x_3^* = 0$. In turn, the latter implies in (3.3*c*) that $x_2^* = 0$. However, (3.3*b*) does not vanish at $(1, 0, 0)$. Therefore, $(1, 0, 0)$ is not an equilibrium. The claim is proven by following similar arguments on the rest of the walls defined in (3.2). □

It follows from Lemma 3.1 that if $x' = G(x, \varepsilon)$ has an equilibrium point for $\varepsilon > 0$, then it is necessarily located in the interior of $\mathcal{C}$. Another property of the cube $\mathcal{C}$ is that it is forward invariant under the flow generated by (2.3). Before proving this statement, we give the definition of a forward invariant set.

Definition 3.4. *Let F be a smooth vector field on $\mathbb{R}^n$, and denote by $\phi(t, z) : \mathbb{R} \times \mathbb{R}^n \to \mathbb{R}^n$ the flow generated by F. We say that a set $\mathcal{S} \subset \mathbb{R}^n$ is forward invariant if $s \in \mathcal{S}$ implies $\phi(t, s) \in \mathcal{S}$ for all $t \geq t_0 \in \mathbb{R}$.*

Lemma 3.2. *For $\varepsilon > 0$, the cube $\mathcal{C}$ is forward invariant under the flow generated by (2.3). Moreover, every trajectory with the initial condition in the boundary of $\mathcal{C}$ evolves towards the interior of $\mathcal{C}$ in forward time.*

Proof. To prove that the cube $\mathcal{C}$ is forward invariant, we need to check the sign of the vector field $G(x,\varepsilon)$ defined by (2.3) restricted to the walls $\mathcal{W}_i$ given in (3.2). It can be readily seen that

$$\begin{aligned} x_1'|_{\mathcal{W}_1} &= -\frac{2x_3}{\varepsilon+2} \leq 0, & x_2'|_{\mathcal{W}_2} &= -\frac{4}{\varepsilon+2} < 0, & x_3'|_{\mathcal{W}_3} &= -\frac{4}{\varepsilon+2} < 0, \\ x_1'|_{\mathcal{W}_4} &= \frac{0.08}{\varepsilon+1} > 0, & x_2'|_{\mathcal{W}_5} &= \frac{8x_1}{\varepsilon+2} \geq 0, & x_3'|_{\mathcal{W}_6} &= \frac{8x_2}{\varepsilon+2} \geq 0. \end{aligned} \tag{3.4}$$

From (3.4) it follows that trajectories of (2.3) cannot leave the cube $\mathcal{C}$, which implies that $\mathcal{C}$ is forward invariant under the flow generated by $G(x,\varepsilon)$. Next, to show our second claim, note that there are three lines (see Figure 2a) where the derivatives in (3.4) may vanish, namely,

$$\ell_1 := \{x \in \mathcal{C} \,|\, x = (x_1, 0, 0)\}, \quad \ell_2 := \{x \in \mathcal{C} \,|\, x = (1, x_2, 0)\}, \quad \ell_3 := \{x \in \mathcal{C} \,|\, x = (0, 0, x_3)\}. \tag{3.5}$$

Let $\phi(t,x) : [t_0,\infty) \times \mathcal{C} \to \mathcal{C}$ denote the forward-flow generated by (2.3). So far, we have shown that for all initial conditions $\phi(t_0,x_0) = x_0 \in \Omega$, where $\Omega = \partial\mathcal{C} \backslash (\ell_1 \cup \ell_2 \cup \ell_3)$, the trajectory $\phi(t,x_0) \in \mathring{\mathcal{C}}$ for all $t > t_0$. Then, we need to check the behavior of the trajectories with those initial conditions on the lines ℓ_1, ℓ_2, and ℓ_3. So, we proceed as follows. The vector field restricted to a line, say ℓ_1, is given by

$$\begin{aligned} x_1' &= \frac{0.08(1-x_1)}{\varepsilon+1-x_1} \\ x_2' &= \frac{8x_1}{\varepsilon+2} \\ x_3' &= 0. \end{aligned} \tag{3.6}$$

From (3.6) we observe that $x_3' = 0$, and $x_2' > 0$ for $x_1 \in (0,1)$. These two facts imply that (3.6) is transversal to the line $\ell_1|_{x_1\in(0,1)}$. Thus, we conclude that a trajectory with an initial condition in $\ell_1|_{x_1\in(0,1)}$ leaves such a line, and hence reaches Ω. Next, consider a trajectory with an initial condition in $\ell_1|_{x_1=0}$. In view of (3.6) one has that $x_1' > 0$ and hence the trajectory is tangent to ℓ_1. This implies that the trajectory reaches the line $\ell_1|_{x_1\in(0,1)}$, which we have discussed above. Similar arguments follow for the lines ℓ_2 and ℓ_3. This completes the proof. □

We now turn to the analysis of the equilibria of (2.3) inside the cube $\mathcal{C}$, i.e., when $x \in \mathring{\mathcal{C}}$. For the case $\varepsilon = 0$, the following lemma is given, whose proof follows from straightforward and standard computations.

Lemma 3.3. *Consider the vector field $x' = G(x,\varepsilon)$ defined by (2.3) with $\varepsilon = 0$. Then the following properties hold:*

- *(i) Let $x \in \mathring{\mathcal{C}}$. Then, the linear algebraic equation $G(x,0) = 0$ has the unique solution $x^0 = (0.5, 0.5, 0.08)$.*
- *(ii) The equilibrium point x^0 is hyperbolic, that is, the Jacobian $D_xG(x,0)|_{x=x^0}$ has eigenvalues with nonzero real parts. Moreover, such eigenvalues satisfy $\lambda_1^0 < 0$ and $\lambda_{2,3}^0 = \alpha^0 \pm i\beta^0$, where $\alpha^0, \beta^0 > 0$.*

Now, assume that $x(\varepsilon) := \left(x_1^\varepsilon, x_2^\varepsilon, x_3^\varepsilon\right)$ is an equilibrium point of (2.3) such that $x(0) = x^0$, where x^0 is the equilibrium point of $x' = G(x,0)$ when $x \in \mathring{\mathcal{C}}$. Linearizing (2.3) at $x(\varepsilon)$ results in $x' = D_xG(x(\varepsilon),\varepsilon)x$ where $D_xG(x(\varepsilon),\varepsilon)$, which denotes the Jacobian matrix calculated at $x(\varepsilon)$, is given by

$$D_xG(x(\varepsilon),\varepsilon) := \begin{bmatrix} \eta_1^\varepsilon & 0 & \theta_1^\varepsilon \\ \theta_2^\varepsilon & \eta_2^\varepsilon & 0 \\ 0 & \theta_3^\varepsilon & \eta_3^\varepsilon \end{bmatrix}, \tag{3.7}$$

with

$$\begin{aligned}
\eta_1^\varepsilon &:= \frac{\partial G_1}{\partial x_1}\Big|_{x=x(\varepsilon)} = -\frac{2\varepsilon x_3^\varepsilon}{(\varepsilon+2x_1^\varepsilon)^2} - \frac{0.08\varepsilon}{(\varepsilon+1-x_1^\varepsilon)^2},\\
\eta_2^\varepsilon &:= \frac{\partial G_2}{\partial x_2}\Big|_{x=x(\varepsilon)} = -\frac{4\varepsilon}{(\varepsilon+2x_2^\varepsilon)^2} - \frac{8\varepsilon x_1^\varepsilon}{(\varepsilon+2(1-x_2^\varepsilon))^2},\\
\eta_3^\varepsilon &:= \frac{\partial G_3}{\partial x_3}\Big|_{x=x(\varepsilon)} = -\frac{4\varepsilon}{(\varepsilon+2x_3^\varepsilon)^2} - \frac{8\varepsilon x_2^\varepsilon}{(\varepsilon+2(1-x_3^\varepsilon))^2},
\end{aligned} \tag{3.8}$$

$$\begin{aligned}
\theta_1^\varepsilon &:= \frac{\partial G_1}{\partial x_3}\Big|_{x=x(\varepsilon)} = \frac{-2x_1^\varepsilon}{\varepsilon+2x_1^\varepsilon},\\
\theta_2^\varepsilon &:= \frac{\partial G_2}{\partial x_1}\Big|_{x=x(\varepsilon)} = \frac{8(1-x_2^\varepsilon)}{\varepsilon+2(1-x_2^\varepsilon)},\\
\theta_3^\varepsilon &:= \frac{\partial G_3}{\partial x_2}\Big|_{x=x(\varepsilon)} = \frac{8(1-x_3^\varepsilon)}{\varepsilon+2(1-x_3^\varepsilon)}.
\end{aligned} \tag{3.9}$$

Remark 3.1. *It is not possible to analytically compute the equilibrium point $x(\varepsilon)$ of (2.3) for $\varepsilon > 0$. To see this, note that one needs to solve simultaneously the equations $G_i(x,\varepsilon) = 0$, $i = 1,2,3$, which results in*

$$0.04(1-x_1)(\varepsilon+2x_1) - (\varepsilon+1-x_1)x_1x_3 = 0, \tag{3.10a}$$

$$2(1-x_2)(\varepsilon+2x_2)x_1 - (\varepsilon+2(1-x_2))x_2 = 0, \tag{3.10b}$$

$$2(1-x_3)(\varepsilon+2x_3)x_2 - (\varepsilon+2(1-x_3))x_3 = 0, \tag{3.10c}$$

each of which is a polynomial of degree 3. If, for example, we solve x_3 from (3.10a) and substitute it in (3.10c), the obtained equation can then be solved for x_2. This solution in turn is substituted in (3.10b) leading to a 9th-degree polynomial of x_1 with ε-dependent coefficients, which from the Abel-Ruffini theorem [13] is impossible to solve analytically. This explains why we use regular perturbation arguments to study (2.3).

From Lemma 3.3 we know that the equilibrium point of $x' = G(x,0)$, when $x \in \mathring{\mathcal{C}}$, is unique and hyperbolic. The following lemma shows that all the *local* properties of x^0 persist under sufficiently small perturbations of ε. That is $x' = G(x,0)$ is structurally stable around x^0, see [11, Theorem 2.2].

Lemma 3.4. *The vector field $x' = G(x,\varepsilon)$ defined by (2.3) has the following properties:*

(i) The Jacobian $D_xG(x,\varepsilon)$ is a smooth function of ε for all $x \in \mathring{\mathcal{C}}$.
(ii) For sufficiently small $\varepsilon > 0$, the equilibrium x^0 perturbs to the unique equilibrium $x(\varepsilon) = x^0 + O(\varepsilon)$, which has the same local stability properties as x^0.

Proof. The nonzero entries of the Jacobian $D_xG(x,\varepsilon)$, defined in (3.7), can be rewritten as an additive combination of terms of the form

$$Q(x,\varepsilon) := \frac{A(x,\varepsilon)}{B(x,\varepsilon)+C(x)}, \tag{3.11}$$

where $A(x,\varepsilon)$, $B(x,\varepsilon)$ and $C(x)$ are polynomials satisfying: i) $B(x,\varepsilon)+C(x) > 0$ for all $x \in \mathcal{C}$ and $\varepsilon > 0$, ii) $B(x,0) = 0$, iii) $C(x) > 0$ for all $x \in \mathring{\mathcal{C}}$, and iv) $C(x) = 0$ if $x \in \partial\mathcal{C}$, that is, $C(x)$ vanishes only in the boundary of $\mathcal{C}$. Thus, to show the first property holds, it suffices to show that (3.11) is a smooth function of ε in $\mathring{\mathcal{C}}$. It is clear that the only point where the k-th derivatives of $Q(x,\varepsilon)$ with respect to ε, i.e. $D_\varepsilon^k Q(x,\varepsilon)$, $k = 0,1,\ldots$, are undefined is whenever $B(x,\varepsilon)+C(x) = 0$. From the above properties $B(x,\varepsilon)+C(x)$ may vanish only when $\varepsilon = 0$. Thus, to ensure that $D_\varepsilon^k Q(x,\varepsilon)$ is well-defined for $\varepsilon \geq 0$, we just need $x \in \mathring{\mathcal{C}}$. Next, the first part of the second property follows from Lemma 3.3, and the implicit function theorem. For the stability properties of $x(\varepsilon)$, it follows from the fact that the eigenvalues of a matrix, depending smoothly on a parameter, vary continuously with respect to such a parameter [11]. □

The characteristic polynomial corresponding to the Jacobian matrix $D_xG(x(\varepsilon),\varepsilon)$, defined in (3.7), is given by

$$P(\lambda,\varepsilon)=\lambda^3+k_1^\varepsilon\lambda^2+k_2^\varepsilon\lambda+k_3^\varepsilon,$$

where

$$k_1^\varepsilon:=-(\eta_1^\varepsilon+\eta_2^\varepsilon+\eta_3^\varepsilon),\qquad k_2^\varepsilon:=\eta_1^\varepsilon\eta_2^\varepsilon+\eta_1^\varepsilon\eta_3^\varepsilon+\eta_2^\varepsilon\eta_3^\varepsilon,\qquad k_3^\varepsilon:=-(\eta_1^\varepsilon\eta_2^\varepsilon\eta_3^\varepsilon+\theta_1^\varepsilon\theta_2^\varepsilon\theta_3^\varepsilon). \tag{3.12}$$

Remark 3.2. *For any $\varepsilon>0$, it follows from Lemmas 3.2 and 3.4, and equations (3.8) and (3.9) that $\eta_i^\varepsilon<0$ $(i=1,2,3)$, $\theta_1^\varepsilon<0$, and $\theta_2^\varepsilon,\theta_3^\varepsilon>0$. Hence, $k_i^\varepsilon>0$.*

The equilibrium $x(\varepsilon)$ is stable when all roots of $P(\lambda,\varepsilon)$ have negative real parts, and unstable if *at least one* of the roots has a positive real part. Applying the Routh-Hurwitz criterion and denoting

$$\Gamma(\varepsilon):=k_1^\varepsilon k_2^\varepsilon-k_3^\varepsilon, \tag{3.13}$$

the following proposition is given for the stability of $x(\varepsilon)$.

Proposition 3.1. *For any $\varepsilon>0$, the equilibrium point $x(\varepsilon)$ is stable if $\Gamma(\varepsilon)>0$, and is unstable if $\Gamma(\varepsilon)<0$.*

Proof. According to the Routh-Hurwitz criterion, the equilibrium point $x(\varepsilon)$ is stable if $k_1^\varepsilon,k_3^\varepsilon,\Gamma(\varepsilon)>0$, and it is unstable if at least one of these conditions is violated. We know from Remark 3.2 that $k_1^\varepsilon,\ k_3^\varepsilon>0$. So, the only quantity that can change the stability of the equilibrium point is $\Gamma(\varepsilon)$. Thus, based on the Routh-Hurwitz criterion, the equilibrium point is stable if $\Gamma(\varepsilon)$ is positive, and it is unstable if it is negative. □

Remark 3.3. *From Remark 3.2 we know that the coefficients of the characteristic polynomial $P(\lambda,\varepsilon)$ are positive $(k_i^\varepsilon>0)$ when $\varepsilon>0$. Therefore, due to the fact that $\det(D_xG(x(\varepsilon),\varepsilon))<0$, one of its roots is negative and the other two are either real of the same sign or complex-conjugated. However, we know from Lemma 3.4 that the eigenvalues of $D_xG(x^0,0)$ satisfy $\lambda_1^0<0$ and $\lambda_{2,3}^0=\alpha^0\pm i\beta^0$, where $\alpha^0,\beta^0>0$. Moreover, from the structural stability of $x'=G(x,0)$ we know that for sufficiently small $\varepsilon>0$, the eigenvalues of $D_xG(x(\varepsilon),\varepsilon)$ satisfy $\lambda_1(\varepsilon)<0$ and $\lambda_{2,3}(\varepsilon)=\alpha(\varepsilon)\pm i\beta(\varepsilon)$ with $\alpha(\varepsilon),\beta(\varepsilon)>0$, where $\lambda_i(0)=\lambda_i^0$, $\alpha(0)=\alpha^0$, and $\beta(0)=\beta^0$.*

What we have studied so far are the local stability properties of the equilibrium point of (2.3) and the forward invariance of $\mathcal{C}$. However, since we are investigating a biochemical oscillator model, one of the most important questions is about the existence of periodic solutions. In particular, it is necessary to describe the relationship between the parameter ε and the existence of and the convergence to such solutions. Furthermore, from Remark 3.3 we know that the equilibrium point $x(\varepsilon)$ has a pair of associated complex-conjugated eigenvalues. This motivates the further analysis via Hopf bifurcation theory, presented in the following section.

4. Hopf bifurcation analysis

In this section we give sufficient conditions for the existence of periodic solutions of (2.3). In principle, the existence of such solutions depends on the parameter ε. We know from Remark 3.3 that $\lambda_1(\varepsilon)<0$ and $\lambda_{2,3}(\varepsilon)=\alpha(\varepsilon)\pm i\beta(\varepsilon)$, with $\alpha(\varepsilon),\ \beta(\varepsilon)>0$, for sufficiently small $\varepsilon>0$. Therefore, upon variation of ε, the eigenvalues $\lambda_{2,3}(\varepsilon)$ may cross transversally the imaginary axis. This would allow us to apply the Hopf bifurcation theorem to prove the existence of periodic solutions. The first step is then to further study the behavior of $\alpha(\varepsilon)$.

Lemma 4.1. *For any $\varepsilon \geq 0$, the real part of $\lambda_{2,3}(\varepsilon)$ satisfies the equation*

$$\Gamma(\varepsilon) = -2\alpha(\varepsilon)[(k_1^\varepsilon + 2\alpha(\varepsilon))^2 + k_2^\varepsilon], \tag{4.1}$$

where $\Gamma(\varepsilon)$ is defined in (3.13).

Proof. Since $P(\lambda, \varepsilon)$ is a cubic function with respect to λ, we may assume without loss of generality that its zeros are $\lambda_1(\varepsilon) \in \mathbb{R}$ and $\lambda_{2,3}(\varepsilon) = \alpha(\varepsilon) \pm i\beta(\varepsilon)$. Based on the Vieta's formulas, the following relations among such zeros hold:

$$\lambda_1^\varepsilon + \lambda_2^\varepsilon + \lambda_3^\varepsilon = -k_1^\varepsilon, \qquad \lambda_1^\varepsilon\lambda_2^\varepsilon + \lambda_1^\varepsilon\lambda_3^\varepsilon + \lambda_2^\varepsilon\lambda_3^\varepsilon = k_2^\varepsilon, \qquad \lambda_1^\varepsilon\lambda_2^\varepsilon\lambda_3^\varepsilon = -k_3^\varepsilon, \tag{4.2}$$

where k_i^ε are defined in (3.12). Then

$$\begin{aligned}
\Gamma(\varepsilon) &= k_1^\varepsilon k_2^\varepsilon - k_3^\varepsilon \\
&= -(\lambda_1^\varepsilon + \lambda_2^\varepsilon + \lambda_3^\varepsilon)k_2^\varepsilon + \lambda_1^\varepsilon\lambda_2^\varepsilon\lambda_3^\varepsilon \\
&= -\lambda_1^\varepsilon(k_2^\varepsilon - \lambda_2^\varepsilon\lambda_3^\varepsilon) - (\lambda_2^\varepsilon + \lambda_3^\varepsilon)k_2^\varepsilon \\
&= -\lambda_1^\varepsilon[2\alpha(\varepsilon)\lambda_1^\varepsilon] - 2\alpha(\varepsilon)k_2^\varepsilon \\
&= -2\alpha(\varepsilon)[(\lambda_1^\varepsilon)^2 + k_2^\varepsilon] \\
&= -2\alpha(\varepsilon)[(k_1^\varepsilon + 2\alpha(\varepsilon))^2 + k_2^\varepsilon].
\end{aligned}$$

□

From Lemma 4.1 and the fact that $k_2^\varepsilon > 0$ (Remark 3.2) we have $\operatorname{sgn}(\Gamma(\varepsilon)) = -\operatorname{sgn}(\alpha(\varepsilon))$ for any $\varepsilon \geq 0$. So, one concludes that if there exists ε^0 such that $\Gamma(\varepsilon^0) = 0$, then the real part of the eigenvalues is zero at ε^0, i.e. $\alpha(\varepsilon^0) = 0$. Therefore ε^0 is the bifurcation point at which the equilibrium point switches from being instable to stable. This change of stability is an important factor towards showing the existence of periodic solutions by means of the Hopf bifurcation theorem [14].

Theorem 4.1 (Hopf bifurcation theorem)**.** *Assume that system $z' = F(z, \mu)$, with $(z, \mu) \in \mathbb{R}^n \times \mathbb{R}$, has an equilibrium point $(z(\mu^0), \mu^0)$ where the vector field F is sufficiently smooth on a sufficiently large open set containing $(z(\mu^0), \mu^0)$. Assume that the following properties hold:*

(i) The Jacobian $D_z F\big|_{(z(\mu^0),\mu^0)}$ has a simple pair of pure imaginary eigenvalues $\lambda(\mu^0)$ and $\overline{\lambda(\mu^0)}$, and the real parts of the other eigenvalues are not zero,

(ii) $\dfrac{d}{d\mu}(Re\,\lambda(\mu))\big|_{\mu=\mu^0} \neq 0.$

Then the dynamics $z' = F(z, \mu)$ undergo a Hopf bifurcation at $(z(\mu^0), \mu^0)$, that is, in a sufficiently small neighborhood of $(z(\mu^0), \mu^0)$, a family of periodic solutions exists.

The following lemma demonstrates the existence of periodic solutions for (2.3) with $\varepsilon > 0$.

Lemma 4.2. *For system* (2.3) *parametrized by $\varepsilon > 0$, there exists $\varepsilon^0 > 0$ such that the dynamics $x' = G(x, \varepsilon)$ undergo a Hopf bifurcation at $(x(\varepsilon^0), \varepsilon^0)$.*

Proof. Recall from Remark 3.3 that for sufficiently small $\varepsilon > 0$, we have $\lambda_1(\varepsilon) < 0$, and the other two eigenvalues are in the form of $\lambda_{2,3}(\varepsilon) = \alpha(\varepsilon) + i\beta(\varepsilon)$. On one hand, it follows from Lemma 3.4 that $\Gamma(\varepsilon)$ is a smooth function of ε. On the other hand, $\Gamma(0) < 0$ and $\Gamma(1) > 0$ (which is computed numerically from Remark 3.1). Therefore, there exists $0 < \varepsilon^0 < 1$ such that $\Gamma(\varepsilon^0) = 0$, which means that the eigenvalues $\lambda_{2,3}(\varepsilon)$ cross the imaginary axis. In Figure 3 we observe that $\Gamma'(\varepsilon^0) > 0$. Due to the latter fact, there exists a neighborhood $\mathcal{N} = (\varepsilon^0 - \delta, \varepsilon^0 + \delta)$, with $\delta > 0$, such that $\Gamma'(\varepsilon) > 0$ for all $\varepsilon \in \mathcal{N}$. In view of Lemma 4.1, one concludes that $\alpha(\varepsilon^0) = 0$. Therefore, $D_x G(x(\varepsilon^0), \varepsilon^0)$ has a

pair of pure imaginary eigenvalues $\pm i\beta(\varepsilon^0)$, and the other eigenvalue is negative (i.e. $\lambda_1(\varepsilon^0) < 0$, Remark 3.3), satisfying assumption (i) of Theorem 4.1.

In addition, the differentiation of (4.1) with respect to ε gives

$$\Gamma'(\varepsilon) = -2\left(\alpha'(\varepsilon)\left[(k_1^\varepsilon + 2\alpha(\varepsilon))^2 + k_2^\varepsilon\right] + \alpha(\varepsilon)\frac{d}{d\varepsilon}\left[(k_1^\varepsilon + 2\alpha(\varepsilon))^2 + k_2^\varepsilon\right]\right). \tag{4.3}$$

Now, due to the fact that $\alpha(\varepsilon^0) = 0$, evaluating (4.3) at $\varepsilon = \varepsilon^0$ results in

$$\Gamma'(\varepsilon^0) = -2\alpha'(\varepsilon^0)\left[(k_1^{\varepsilon^0})^2 + k_2^{\varepsilon^0}\right], \tag{4.4}$$

and hence

$$\alpha'(\varepsilon^0) = \frac{\Gamma'(\varepsilon^0)}{-2\left[(k_1^{\varepsilon^0})^2 + k_2^{\varepsilon^0}\right]}. \tag{4.5}$$

Note that from Remark 3.2 we know that $k_1^{\varepsilon^0}, k_2^{\varepsilon^0} > 0$, and hence (4.5) is well-defined. Recalling $\Gamma'(\varepsilon^0) > 0$, one concludes that $\alpha'(\varepsilon^0) < 0$, and hence the second assumption of Theorem 4.1 is satisfied. Therefore, the dynamics $x' = G(x, \varepsilon)$ undergo a Hopf bifurcation at $(x(\varepsilon^0), \varepsilon^0)$. □

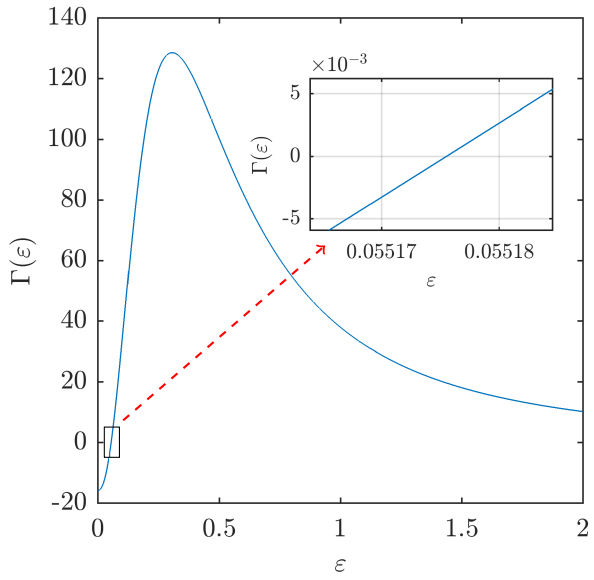


Figure 3: The curve Γ with respect to ε, and the zoom-in of Γ near the bifurcation value $\varepsilon = \varepsilon^0$.

From Lemma 4.2 we know that system (2.3) undergoes a Hopf bifurcation at $(x(\varepsilon^0), \varepsilon^0)$. The numerical continuation software MATCONT [15] is used to track such a bifurcation. The value of the bifurcation parameter, computed by MATCONT, is $\varepsilon^0 \simeq 0.05517665$. The equilibrium point corresponding to ε^0 is $x(\varepsilon^0) = (0.48668602, 0.37822906, 0.07633009)$. The bifurcation diagrams of x_1, x_2 and x_3 with respect to ε, and their zoom-ins around the Hopf bifurcation point "H" are presented in Figures (4a), (4b) and (4c), respectively. In Figure 4, the black curves depict the position of the equilibrium point $x(\varepsilon)$; the dashed black curve corresponds to the case when $x(\varepsilon)$ is unstable, while the solid one represents the case when $x(\varepsilon)$ is stable. On the other hand, the red and blue curves correspond to periodic solutions; the solid blue curve indicates that the periodic solution is stable, while the dashed red one shows that the periodic solution is unstable. For each fixed ε, these curves provide the maximum and the minimum values of each variable along the corresponding periodic solution. Moreover, in Figures (4a), (4b) and (4c) in the zoom-ins around

the Hopf bifurcation point "H", we observe that for a range of ε started from ε^0, both stable and unstable periodic solutions exist simultaneously.

In this section we have shown the existence of periodic solutions in (2.3) for $\varepsilon \in (0, \varepsilon^0)$. However, the presented results do not consider the stability of the periodic solutions. Furthermore, the number of periodic solutions is still unknown. These issues are treated in the following section.

5. Global behavior of solutions

The local analysis performed in the previous section does not fully capture the behavior of the solutions of (2.3). For example, we cannot conclude directly from the previous results whether the trajectories are oscillatory or they evolve in some unexpected way, e.g. chaotically. In this section, we show that when the equilibrium point $x(\varepsilon)$ of (2.3) is unstable, *almost all* trajectories converge to periodic solutions, ruling out chaotic behavior and the existence of homoclinic solutions. To this end, we study the structure of the ω-limit set of (2.3). We start with the following definition.

Definition 5.1. *For a function $z : [t_0, \infty) \to \mathbb{R}^m$, where $t_0 \in \mathbb{R}$, a point z^* is called an ω-limit point if there exists a sequence $\{t_n\}$, $t_n \xrightarrow[n\to\infty]{} \infty$, such that $z(t_n) \xrightarrow[n\to\infty]{} z^*$; the set of all ω-limit points is referred to as the ω-limit set of the function $z(\cdot)$ and denoted by $\omega(z)$.*

In general, an ω-limit set can be empty. However, if a function is *bounded*, then its ω-limit set is nonempty, closed and connected [16]. In the context of system (2.3), the ω-limit set of a trajectory with an initial condition in $\mathcal{C}$ is non-empty due to the forward invariance of $\mathcal{C}$ (see Lemma 3.2).

For *planar* autonomous dynamical systems, the structure of the ω-limit set of solutions is given by the celebrated Poincaré-Bendixson theorem. This theorem states that the ω-limit set of a bounded solution is either (i) an equilibrium point, (ii) a closed trajectory, or (iii) the union of equilibria and the trajectories connecting them [12]. The latter are referred to as *heteroclinic* solutions when they connect distinct points, and *homoclinic* solutions when they connect a point to itself. Although the Poincaré-Bendixson theorem is not applicable to systems of dimensions higher than 2, it holds for *monotone* cyclic feedback systems [17]. For the reader's convenience, we formulate the results of [17] on the positively invariant domain $\mathbb{R}^n_+ := [0, \infty)^n$ with bounded solutions as follows.

Consider a system of the form

$$y_i' = f_i(y_{i-1}, y_i), \qquad i = 1, 2, ..., n, \tag{5.1}$$

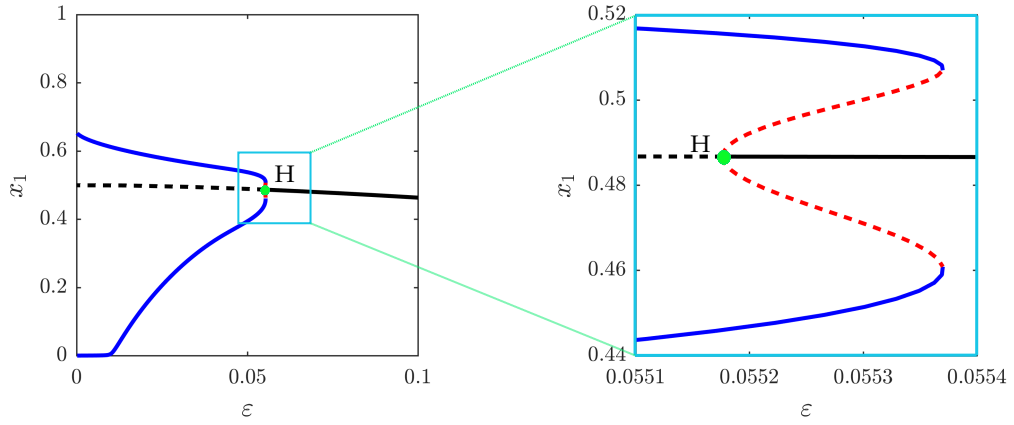
where y_0 is interpreted as y_n, and the nonlinearity $f = (f_1, f_2, ..., f_n)$ is assumed to be C^1-smooth on $\mathbb{R}^n_+$. Systems of the form (5.1) are called cyclic. The *fundamental* assumption on (5.1) is that the variable y_{i-1} influences f_i *monotonically*. So, it is assumed that for some $\delta_j \in \{-1, 1\}$, the conditions

$$\delta_i \frac{\partial f_i(y_{i-1}, y_i)}{\partial y_{i-1}} > 0, \qquad i = 1, 2, ..., n, \tag{5.2}$$

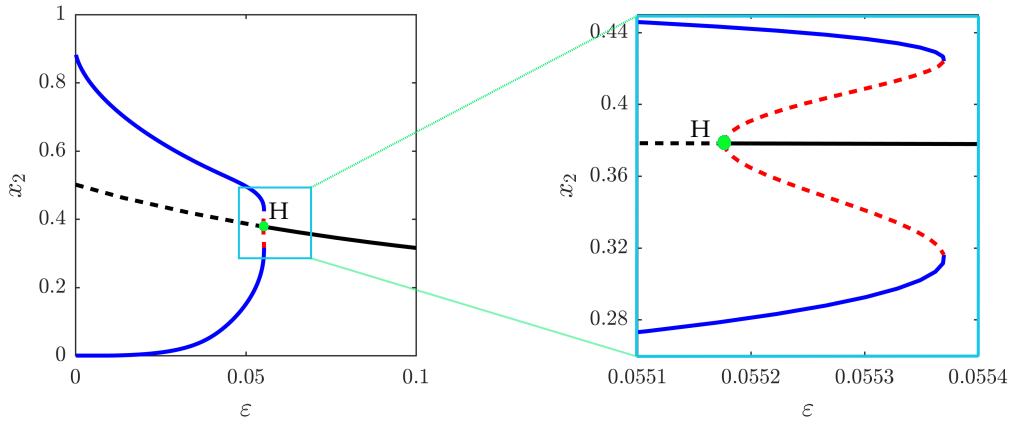
hold, meaning that the functions f_i are *strictly* monotone in y_{i-1}. Moreover, δ_i describes whether the role of y_{i-1} is to reduce ($\delta_i = -1$) the growth of y_i, or to augment ($\delta_i = 1$) it. The product

$$\Delta := \prod_{i=1}^{n} \delta_i \tag{5.3}$$

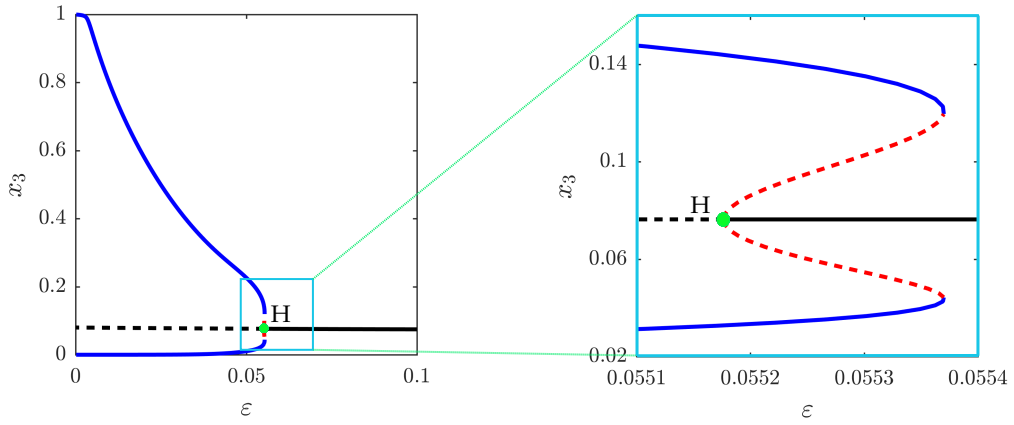
describes whether the entire system has positive feedback ($\Delta = 1$) or negative feedback ($\Delta = -1$). A cyclic system (5.1) that satisfies conditions (5.2) is called a *monotone cyclic feedback system*, and it is shown in [17] that they have the Poincaré-Bendixson properties. We recall this important result in Theorem 5.1. Before that, we give the following definitions.



(a)



(b)



(c)

Figure 4: In the left-side of Figures (a), (b), and (c), we show the bifurcation diagrams of x_1, x_2 and x_3 with respect to ε, whose zoom-ins around the Hopf bifurcation point, denoted by "H", are given on the right-side. The black curve depicts the position of the unique equilibrium point $x(\varepsilon)$; the dashed (resp. solid) section of this curve represents the interval within which $x(\varepsilon)$ is unstable (resp. stable). The solid blue and the dashed red curves describe the amplitude of oscillation for each of the variables. The solid blue curve corresponds to a stable periodic solution, while the dashed red curve indicates an unstable one.

Definition 5.2. *[18] Assume that $z^p(t)$ is a periodic solution for the dynamical system $z' = F(z) \in \mathbb{R}^n$. The solution $z^p(t)$ is said to be* orbitally stable *if for each $\epsilon > 0$, there exists a corresponding $\delta > 0$ such that every solution $z(t)$ of $z' = F(z)$, whose distance from $z^p(t)$ is less than δ for $t = t_0$, is defined and remains at a distance less than ϵ from $z^p(t)$ for all $t \geq t_0$. Moreover, if the distance of $z(t)$ from $z^p(t)$ tends to zero as $t \longrightarrow \infty$, the periodic solution $z^p(t)$ is called* orbitally asymptotically stable.

Definition 5.3. *The distance between two sets $\mathcal{S}_1, \mathcal{S}_2 \subset \mathbb{R}^n$ is denoted and defined by*

$$d(\mathcal{S}_1, \mathcal{S}_2) := \inf\{\|s_1 - s_2\| \ : \ s_1 \in \mathcal{S}_1, s_2 \in \mathcal{S}_2\}, \tag{5.4}$$

where $\|\cdot\|$ is an arbitrary norm in $\mathbb{R}^n$.

Definition 5.4. *[19] Let F be a smooth vector field on $\mathbb{R}^n$ and denote by $\phi(t, z) : \mathbb{R} \times \mathbb{R}^n \to \mathbb{R}^n$ the flow generated by F.*

- *A set $\mathcal{K} \subset \mathbb{R}^n$ is said to* attract *a set $\mathcal{M} \subset \mathbb{R}^n$, if $\mathcal{K} \neq \emptyset$ and $d(\mathcal{K}, \phi(t, \mathcal{M})) \to 0$ as $t \to \infty$. We also say that $\mathcal{M}$ is attracted by $\mathcal{K}$.*
- *A set $\mathcal{K}$ is called an* attractor *of $\mathcal{M}$, if $\mathcal{K}$ is invariant and attracts $\mathcal{M}$. In this situation, we also say that $\mathcal{M}$ has the attractor $\mathcal{K}$. The set $\mathcal{K}$ is called a* compact attractor *of $\mathcal{M}$ if, in addition, $\mathcal{K}$ is compact.*

Next, for brevity, we recall the relevant results of Theorems 4.1 and 4.3 of [17] as follows.

Theorem 5.1. *Let the cyclic system (5.1) satisfy conditions (5.2) in $\mathbb{R}^n_+$. Then the following statements hold.*

(i) Assume that $\mathbb{R}^n_+$ is forward invariant for (5.1), and that it contains a unique equilibrium point y^*. Then the structure of the ω-limit set of any bounded solution of the system is either

(a) the equilibrium point y^*,
(b) a nonconstant periodic solution, or
(c) the equilibrium point y^* together with a collection of solutions homoclinic to y^*. This case does not occur if

$$\Delta \det(-D_y f(y^*)) < 0, \tag{5.5}$$

where $D_y f(y^*)$ denotes the Jacobian matrix of system (5.1) at y^*.

(ii) Suppose that (5.1) satisfies $\Delta = -1$, and possesses a compact attractor $\mathcal{K} \subset \mathring{\mathbb{R}}^n_+$. Assume that $\mathcal{K}$ contains a unique equilibrium point y^*, and that $D_y f(y^*)$ satisfies (5.5) and has at least two eigenvalues with positive real parts. Then (5.1) has at least one, but no more than a finite number of nontrivial periodic solutions. Moreover, at least one of such solutions is orbitally asymptotically stable.

Remark 5.1. *[17] In Theorem 5.1, $\mathbb{R}^n_+$ can be replaced by any other forward invariant closed* convex *domain Ω containing a single equilibrium point.*

Now, we describe the global behavior of solutions of system (2.3) as follows.

Theorem 5.2. *For sufficiently small $\varepsilon > 0$, and for almost all initial conditions $x_0 \in \mathcal{C}$, the trajectories $\phi(t, x_0)$ of (2.3) converge to a finite number of nonconstant periodic solutions. Moreover, at least one of such solutions is orbitally asymptotically stable.*

Proof. First of all, to show that (2.3) is cyclic, note that it can be written as

$$x_1' = G_1^\varepsilon(x_3, x_1),$$
$$x_2' = G_2^\varepsilon(x_1, x_2),$$
$$x_3' = G_3^\varepsilon(x_2, x_3).$$

Thus, system (2.3) is cyclic for any ε. Next, recalling Remark 3.2, we have that $\frac{\partial G_1^\varepsilon}{\partial x_3} < 0$, $\frac{\partial G_2^\varepsilon}{\partial x_1} > 0$, and $\frac{\partial G_3^\varepsilon}{\partial x_2} > 0$, which implies that, according to (5.2) and (5.3), $\delta_1 = -1$, $\delta_2 = \delta_3 = 1$ and hence $\Delta = -1$. This means that (2.3) is a monotone cyclic negative feedback system. In view of $\det(D_x G(x(\varepsilon), \varepsilon)) < 0$ (Remark 3.3), we conclude that (2.3) satisfies (5.5). Therefore, from statement (i) of Theorem 5.1, the ω-limit set of any trajectory of (2.3) with the initial condition $x_0 \in \mathcal{C}$ is either an equilibrium point or a nonconstant periodic solutions. Then, recall from our local analysis results in Lemma 3.4 that for sufficiently small $\varepsilon > 0$, the equilibrium point $x(\varepsilon)$ is associated with a 1-dimensional stable and a 2-dimensional unstable manifolds. This means that the only trajectories that converge to the equilibrium point $x(\varepsilon)$ are those with the initial conditions along the stable manifold, while all the other trajectories, due to the above arguments, converge to some non-constant periodic solution. Note that the set of initial conditions contained in the stable manifold is negligible[1] with respect to all other initial conditions in $\mathcal{C}$.

Next, due to the fact that the cube $\mathcal{C}$ is forward invariant for any $\varepsilon > 0$ (Lemma 3.2), system (2.3) possesses a compact attractor $\mathcal{K} \subset \mathring{\mathcal{C}}$ [17]. Moreover, for sufficiently small $\varepsilon > 0$ and from Lemmas 3.3 and 3.4, we know that the equilibrium point $x(\varepsilon)$ is unique, the Jacobian matrix $D_x G(x(\varepsilon), \varepsilon)$ has two eigenvalues with positive real parts, and $\Delta \det(-D_x G(x(\varepsilon), \varepsilon)) < 0$. Therefore, system (2.3) satisfies all the assumptions of the statement (ii) of Theorem 5.1, and hence (2.3) has a finite number of non-constant periodic solutions, at least one of which is orbitally asymptotically stable. □

Remark 5.2. *From the bifurcation analysis performed in Section 4, it is clear that by "for sufficiently small $\varepsilon > 0$" in Theorem 5.2 we mean $\varepsilon \in (0, \varepsilon^0)$.*

6. On the robustness of bifurcation with respect to parameter changes

This section is devoted to investigate how robust our bifurcation analysis and qualitative results are under small but not necessarily symmetric changes in the parameters of system (2.3). Note that our bifurcation analysis is based only on the scalar parameter ε, because, as discussed in Section 2, we have unified all the Michaelis-Menten constants by ε, i.e., $K_{\mathrm{a}} = 2K_{\mathrm{d}} = 2K_{\mathrm{m}} = 2K_{\mathrm{dm}} = 2K_{\mathrm{p}} = 2K_{\mathrm{dp}} = \varepsilon$. Now, we are interested in understanding how the conclusion of the bifurcation analysis may change if there is a small "asymmetry" in parameter values. In other words, we want to know how system (2.3) behaves if the perturbation of the parameters is no longer restricted to the scalar parameter ε, but depends on a 6-dimensional parameter vector according to the Michaelis-Menten constants.

Claim 6.1. *The bifurcation analysis result for $G(x, \varepsilon) = 0$, given by (2.4), is robust in the sense that any (smooth, sufficiently small, and not necessarily symmetric) change in the parameters will lead to the same qualitative behavior of the solutions as that already described in this paper.*

To provide a formal proof of Claim 6.1 (see Proposition 6.1 below) we follow [20]. To avoid making this section inconveniently long, we adopt the same terminology and notation as in [20] and recall just the essential definitions and results. For more details on the concepts being used below, and a brief introduction to algebraic geometry and singularity theory, the interested reader is referred to [20] and [21] respectively.

[1] A subset of Euclidean space is called negligible if its Lebesgue measure is zero.

Let G be a (germ of a) function in $n+1$ variables near 0, that is $G:(\mathbb{R}^n\times\mathbb{R},0)\to(\mathbb{R}^m,0)$.

Definition 6.1. *[20, Definition 2.1a] An ℓ-parameter unfolding of G is a C^∞ map $F:(\mathbb{R}^n\times\mathbb{R}\times\mathbb{R}^\ell,0)\to(\mathbb{R}^m,0)$ such that $F(x,\lambda,0)=G(x,\lambda)$ for all $(x,\lambda)\in\mathbb{R}^n\times\mathbb{R}$.*

Definition 6.2. *[20, Definition 2.1c] F is a universal unfolding of G if every unfolding of G factors through F.*

In some sense, a bifurcation problem defined by $F=0$ contains all the qualitative behavior present in $G=0$. Moreover, any other unfolding of G does not contain new information or behavior already given by F. Thus, the goal is, given a bifurcation problem $G=0$, to know if a universal unfolding F exists, and if it does, to compute it.

In order to address the aforementioned issue, let us first introduce some notations: we denote an ℓ-parameter unfolding of G by F_α with some fixed $\alpha\in\mathbb{R}^\ell$. We denote by $\mathcal{E}_{n+1}$ the ring of germs of (smooth) functions in n-variables and 1-parameter $(x,\lambda)\in\mathbb{R}^n\times\mathbb{R}$, and regard $\mathcal{E}_{n+1}^m$, the space of m-tuples, as a module over $\mathcal{E}_{n+1}$ with component-wise multiplication. Moreover, we denote by $\mathcal{E}_{n+1}\left\{\frac{\partial G}{\partial x}\right\}$ the submodule of $\mathcal{E}_{n+1}^m$ generated by $\partial G/\partial x_1,\partial G/\partial x_2,...,\partial G/\partial x_n$ over the ring $\mathcal{E}_{n+1}$, the ideal $\langle G\rangle=\langle G_1,G_2,...,G_m\rangle$ in $\mathcal{E}_{n+1}$ generated by the m components of G, and $\mathcal{E}_\lambda\left\{\frac{\partial G}{\partial\lambda}\right\}:=\left\{\phi(\lambda)\frac{\partial G}{\partial\lambda}\mid\phi\in\mathcal{E}_\lambda\right\}$, where $\phi\in\mathcal{E}_\lambda$ stands for $\phi\in\mathcal{E}_{n+1}$ when ϕ is just a function of λ and does not depend on x.

Remark 6.1. *Recall from Lemma 4.2 that the bifurcation point of (2.4) is $(x(\varepsilon^0),\varepsilon^0)$. Therefore, for the particular bifurcation problem given by (2.4), the ring of germs $\mathcal{E}_{n+1}$ is defined around $x(\varepsilon^0)$ and $\lambda=\varepsilon-\varepsilon^0$ with $n=3$, $m=3$.*

Definition 6.3. *[20, Definition 2.3]*

(i) Let $\tilde{T}G=\langle G\rangle^m+\mathcal{E}_{n+1}\left\{\frac{\partial G}{\partial x}\right\}$ and let $TG=\tilde{T}G+\mathcal{E}_\lambda\left\{\frac{\partial G}{\partial\lambda}\right\}$.

(ii) G has finite codimension if $\dim\left(\mathcal{E}_{n+1}^m/\tilde{T}G\right)<\infty$.

(iii) The codimension of G equals $\dim\left(\mathcal{E}_{n+1}^m/TG\right)$ and is denoted by $\operatorname{codim}G$.

Now, we are ready to present the main result of [20].

Theorem 6.1. *[20, Theorem 2.4] Suppose G has finite codimension, and let F_α be an ℓ-parameter unfolding of G. F_α is a universal unfolding of G if and only if TG plus the ℓ-vectors $\partial F/\partial\alpha_1|_{\alpha=0},\cdots,\partial F/\partial\alpha_\ell|_{\alpha=0}$ together span $\mathcal{E}_{n+1}^m$ (over the reals). The minimum number of unfolding parameters in any universal unfolding is the codimension of G.*

In words, Theorem 6.1 states that given a bifurcation problem G of a certain codimension, say p, we need to add p parameters to the idealized problem $G=0$ to obtain a robust bifurcation problem $F_\alpha=0$. Then, any smooth perturbation whatsoever of the idealized problem $G=0$ will give a qualitative behavior already presented for $F_\alpha=0$.

Now we turn to check whether the bifurcation problem G given in (2.4) is robust.

Proposition 6.1. *The bifurcation problem G in (2.4) has codimension zero, i.e. $\operatorname{codim}G=0$.*

Proof. First of all, note that up to relabeling of the variables (x_1,x_2,x_3), the equations $G_i(x,\varepsilon)=0$, $i=1,2,3$, are all equivalent, where $G_i(x,\varepsilon)$ are the right-hand sides of (2.3). Thus, without loss of generality, we can study, for instance, a bifurcation problem defined by $F=0$, where

$F(x_1, x_2, x_3, \varepsilon) : \mathbb{R}^3 \times \mathbb{R} \to \mathbb{R}$ is given by

$$F(x_1, x_2, x_3, \varepsilon) = \kappa_1\varepsilon + \kappa_2 x_1 + \kappa_3\varepsilon x_1 + \kappa_4 x_1^2 + \kappa_5 x_1 x_3\varepsilon + \kappa_6 x_1 x_3 + \kappa_7 x_1^2 x_3, \tag{6.1}$$

which is the numerator of $G_1(x, \varepsilon)$ where κ_j $(j = 1, 2, ..., 7)$ are non-zero real constants, and we set $m = 1$.

Due to the dimension order $0 \leq \dim(\mathcal{E}_{n+1}/TF) \leq \dim\left(\mathcal{E}_{n+1}/\tilde{T}F\right)$, it suffices to show that $\dim\left(\mathcal{E}_{n+1}/\tilde{T}F\right) = 0$. The quotient space $\dim\left(\mathcal{E}_{n+1}/\tilde{T}F\right)$, its base, and its dimension are computable by hand. However, to simplify such tasks we have used the software "SINGULAR" [22] with which we can automate the necessary computations. By doing so we obtain

$$\dim\left(\mathcal{E}_{n+1}/\tilde{T}F\right) = 0. \tag{6.2}$$

Due to the dimension order $0 \leq \dim(\mathcal{E}_{n+1}/TF) \leq \dim\left(\mathcal{E}_{n+1}/\tilde{T}F\right)$, we conclude that $\dim(\mathcal{E}_{n+1}/TG_1) = \dim(\mathcal{E}_{n+1}/TF) = 0$. As mentioned above, the same claim holds for G_2 and G_3, that is $\dim(\mathcal{E}_{n+1}/TG_2) = \dim(\mathcal{E}_{n+1}/TG_3) = 0$. Thus, from the definition of $\mathcal{E}^m_{n+1}$ it follows that $\dim\left(\mathcal{E}^3_{n+1}/TG\right) = 0$. Therefore $\operatorname{codim} G = 0$. □

Remark 6.2. *The proof of claim 6.1 follows from Theorem 6.1 and Proposition 6.1. As a consequence, the convergence result presented in Theorem 5.2 is robust, in the sense that any small change in the parameters leads to the same qualitative behavior of the solutions.*

7. Conclusions and discussions

In this paper we have studied a biochemical oscillator model that describes the developmental process of myxobacteria. Such an oscillator is proposed in [8] as the control mechanism of motion reversals. With the results of this paper we have formalized and refined the claims made in [8]. Particularly, we have given an estimate of the parameter values ε for which almost all trajectories of the biochemical oscillator indeed converge to a periodic solution.

Our studies start from the local behavior of the biochemical oscillator and conclude with a global description. First of all, we have identified the parameters of the model using a single ε. Then we have developed local analysis through which we have found a unique hyperbolic equilibrium point associated with the oscillator. Since such a point is hyperbolic, it turns out that the system is structurally stable in a small neighborhood of it, motivating us to further investigate the robustness of the system. However, up to this stage, oscillatory behavior cannot yet be explained. So we have used Hopf bifurcation theory to give sufficient conditions for the existence of periodic solutions. From bifurcation analysis we have been able to provide numerical estimates of the range of parameter under which periodic solutions exist. However, the results from the Hopf bifurcation analysis do not provide information on the cardinality of and convergence to periodic solutions. In this regards, we have performed global analysis to show that the number of possible limiting periodic solutions is finite and that trajectories converge to at least one of such solutions. At the end, we have shown that the bifurcation results reported in this paper are robust in the sense that any smooth, sufficiently small, and not necessarily symmetric change in the parameters will lead to the same qualitative behavior of the solutions as the one that has been already described. All these results lead us to conclude that the biochemical oscillator proposed in [8] is indeed robust under sufficiently small C^1-perturbations of the parameter. We emphasize that the presented approach is not confined to the specific oscillator that is studied in this paper, and that the ideas provided here may be applied to other oscillatory systems such as [23].

For future research, we are interested in a couple of open problems. First, Theorem 5.2 shows the convergence of almost all trajectories to a finite number of periodic solutions. However, from simulations it appears that almost all trajectories actually converge to a *unique* limit cycle. The first open problem is to prove this rigorously. Second, from numerical simulations, it is clear that there are several *timescales* along the limit cycle, which are related to the small parameter

ε. A thorough analysis of such timescales and their influences on the dynamics may provide a better understanding of their role in the biochemical clock. Thus, the second open problem is to investigate the model studied in this paper from a multi-timescales perspective.

Data Accessibility. This paper contains no experimental data. All computational results are reproducible.

Authors' Contributions. H.T., H.J.K., and M.C. designed research, performed research, and wrote the paper.

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